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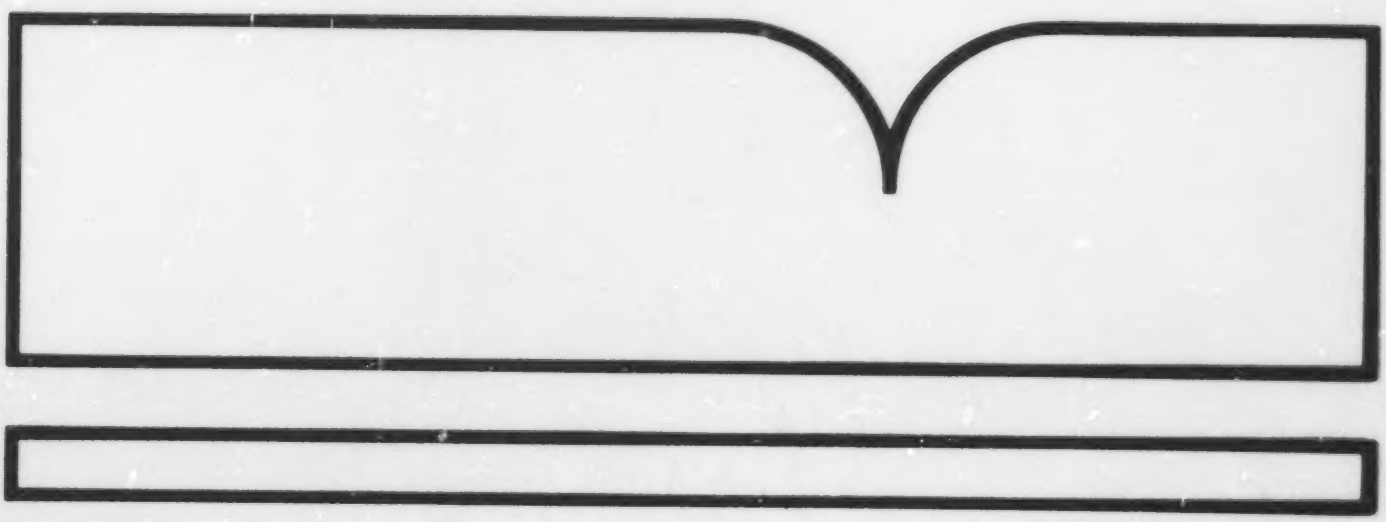

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16. ABSTRACT

In a greenhouse experiment, Malus hupehensis(Pamp.)Rehd. seedlings were treated weekly with simulated acid rain solutions ranging from pH 2.25 to pH 7.0. Necrotic lesions developed on leaves at pH 2.25 and pH 2.50 immediately after the first application at the 8-node stage. Following the 9th weekly application on seedlings with 23 to 26 nodes, lesions developed at pH levels up to 3.25. At final destructive harvest, 20% of the leaf area at pH 2.25 and 8% of the leaf area at pH 2.50 was injures. Significant growth reduction occurred at these 2 pH levels. Regression analysis indicated extensive growth inhibition at pH 3.0, no growth inhibition around pH 3.5, some inhibition between pH 4.5 and pH 5.6, and normal growth at pH 7.0 in comparison to the unsprayed control. Growth was negatively correlated with lesion formation at 3 destructive harvest dates. Relative growth rates were reduced only at pH 2.25 and pH 2.50 and reduction in the unit leaf rate was also observed. Lesion development continued to increase on the basal leaves through the 6th weekly application but leveled off during the final applications. Negative correlations of photosynthesis rate to lesion percentage and dry weight to lesion percentage were observed.

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JOURNAL ARTICLE

Growth Changes of Apple Seedlings in Response to Simulated Acid Rain[1,2]

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Abstract. **In a greenhouse experiment, *Malus hupehensis* (Pamp.) Rehd. seedlings were treated weekly with simulated acid rain solutions ranging from pH 2.25 to pH 7.0. Necrotic lesions developed on leaves at pH 2.25 and pH 2.50 immediately after the first application at the 8-node stage. Following the 9th weekly application on seedlings with 23 to 26 nodes, lesions developed at pH levels up to 3.25. At final destructive harvest, 20% of the leaf area at pH 2.25 and 8% of the leaf area at pH 2.50 was injured. Significant growth reduction occurred at these 2 pH levels. Regression analysis indicated extensive growth inhibition at <pH 3.0, no growth inhibition around pH 3.5, some inhibition between pH 4.5 and pH 5.6, and normal growth at pH 7.0 in comparison to the unsprayed control. Growth was negatively correlated with lesion formation at 3 destructive harvest dates. Relative growth rates were reduced only at pH 2.25 and pH 2.50 and reduction in the unit leaf rate was also observed. Lesion development continued to increase on the basal leaves through the 6th weekly application but leveled off during the final applications. Negative correlations of photosynthesis rate to lesion percentage and dry weight to lesion percentage were observed.**

During the period 1971 to 1981, average acidity of rain increased from ≥ pH 5 to ≤ pH 4 in many areas of northern Europe and the eastern United States (16, 18). This change has been attributed to increased combustion of fossil fuels resulting in wide dispersal of sulfur and nitrogen oxides which are converted to corresponding strong acids in the atmosphere (17).

Crop response to acid precipitation varies with species (4, 15). At pH levels between 4 and 3 many effects, both positive and negative, have been demonstrated. At pH below 3, the chances of harmful effects on growth or yield were substantially increased (12). Soybean yield reduction has been observed at acidity levels as high as pH 4.0 with greater reductions in yield at pH 3.1 and pH 2.7 (5). These yield reductions occurred in the absence of foliar lesions. Some experiments reported a yield increase (growth stimulation) at pH 3–4 for some crops (13, 20). There was no direct evidence that acidity levels of current rainfalls injured field or forest plant foliage. Simulation experiments at low pH, however, have indicated that acid rain leaf necrosis can be induced. Most injury to foliage by simulated acid rain occurred on expanding or recently expanded leaves. Young birch seedlings were much more sensitive than older seedlings (22). Leaves that had just reached full expansion were more sensitive to simulated acid rain than unexpanded and already fully expanded birch leaves (22). Applications of acid rain also inhibited leaf expansion (5). More research is needed to determine the relationship between visible foliar injury and plant growth and/or yield (4). Soluble solids reduction in 'Concord' grape berries were related in part to leaf injury from acid rain at pH 2.5 (8). Decreases in weight and height of sugar maple and yellow birch seedlings at pH 2.3 were observed (6, 21, 22). Additional growth reduction and stomatal disfunction were observed with simulated acid rain in *Phaseolus vulgaris* (7). The least acidic experimental treatment at which visible lesions on leaves have been observed was near pH 4.0 (14). A large percentage of the leaf area may exhibit lesions at pH 3.1 but at levels above 3.1, less than 5% injury was observed (3, 4, 7, 21). Research has shown that foliage of broadleaved herbaceous plants was more sensitive to acid rain than that of broadleaf woody plants or conifers (4, 10, 14). Lesion development has been reported on *Betula* at pH 2.8 (19, 21) and on *Poplar* at pH 2.7 (3).

The pH levels of ambient rain did not affect fruit quality or production in grapes and apples. However, reductions of pollen germination, fruit set, and fruit quality were observed below pH 3.1. Many of these effects were cultivar-specific and yield was not affected by acid rain as low as pH 2.5 (8, 9).

The objective of this research was to determine the simulated acid rain threshold level for lesion development and growth reduction in apple seedlings, including the effect of acid rain on photosynthesis.

Materials and Methods

Various acid rain solutions are used by different researchers in the Northeast (11), the background ion contents varying to match local input conditions. For our experiments, local precipitation chemistry data were obtained from Warren Knapp, Cornell University. For our experiments, acid rain solutions were mixed and sprayed as previously described (8, 9).

After an initial experiment in a greenhouse from June to September 1981, in which inadequate plant populations were available for proper growth analysis, the experiment reported here was begun on Sept. 1, 1981. On that date, 800 *Malus hupehensis* pregerminated seeds were planted in separate 10-cm pots using 'Peat-lite Mix A' (1). *M. hupehensis* seed was selected because of its apomictic origin which gives rise to genetically uniform plants. The 504 most uniform plants were selected for treatment, arranged in a randomized complete block design, and sprayed

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weekly with the following solutions: pH 7.0, 5.6, 4.5, 4.0, 3.75, 3.5, 3.25, 3.0, 2.75, 2.5, and 2.25. A control received no spray. Treatments of pH 7.0 and pH 5.6 served as sprayed controls, with pH 7.0 being the neutral treatment and pH 5.6 being the acidity of naturally occurring rain acidified only by atmospheric CO_2. Solutions were applied initially to seedlings at the 8-node stage (8 unfolded leaves). Foliage was kept wet continuously for 9 hr on each weekly application with 6 spray applications, each 1.5 hr apart. Seedlings were grown in the greenhouse, but supplemental fluorescent and incandescent light at 300 $\mu E\ m^{-2}s^{-1}$ (photosynthetically active radiation, PAR) for a 16-hr photoperiod was provided. Radiation with supplemental light plus sunlight through the shaded greenhouse was 550 $\mu E\ m^{-2}s^{-1}$ PAR. Sprays were applied using plastic hand-pumped sprayers as previously described (8, 9). Flow was adjusted so that foliage was not mechanically harmed by high spray intensities, and droplet sizes were maintained so that no mist was formed that could drift to neighboring plants.

After 3, 6, and 9 weeks, 14 seedlings from each treatment were destructively harvested and the following measurements made: a) fresh and dry weight—total, leaves, shoots, and roots; b) leaf area; c) shoot length; and d) leaf number.

On Nov. 5, 1981, photosynthesis rates were measured on selected leaves from the 7th, 8th or 9th nodes of 15 seedlings after 5 acid rain applications. Three plants from each of 5 treatments (pH 2.25, 2.5, 3.0, 3.25, and 5.6) were selected as these treatments represented a range of lesion severity. Measurements were made with an open gas analysis system using a thin, open-top, clamp-type leaf chamber in a flow system with an infrared gas analyzer. The standard conditions of measurement were: 800 $\mu E\ m^{-2}s^{-1}$ PAR, 25 ± 1 C, 15 ± 2mb vapor pressure deficit, and 1.5 l min^{-1} flow rate. Correlations between leaf lesions and net photosynthesis were calculated. Relative growth rates and unit leaf rates were determined according to the method of Evans (2).

Results and Discussion

Necrotic lesion development on *M. hupehensis* leaves was observed the day after initial acid rain application at pH 2.25 and pH 2.50. By the 2nd and 3rd harvests, i.e., 6 to 9 spray applications, some lesions were observed on leaves treated with solutions as high as pH 3.25 (Table 1). Lesion development on single leaves at node 8 after 9 applications of acid rain at pH 2.25 revealed that 30–35% of the leaf surface was injured. Acid rain at pH 2.25 induced necrosis on 20% of the leaf area of entire seedlings (Table 1). Growth curves on a logarithmic scale are displayed for 3 of the treatments (Fig. 1). For the sprayed control (pH 7.0), the curve was nearly logarithmic as expected (2) up to 42 days, with deviation between 42 and 63 days. This deviation from logarithmic rate may be partially explained by lower natural light levels during the later period into November and December. Greater deviation from the logarithmic trend in treatments pH 2.25 and 2.50 was due to acid rain stress. Plants stressed at pH 2.25 grew nearly linearly between the 21- to 63-day period.

Fig. 2 and Fig. 3 show the time-course development of acid rain lesions on seedlings treated with acid rain at pH 2.25 and pH 2.50. Time interval of new leaf production is observed along with schedule of sprays and assays. At pH 2.25, lesions continued to develop on lower nodes up to the 6th spray application, but between the 6th and 9th applications, leaves on lower nodes showed no further lesion development (Fig. 2). At pH 2.50 (Fig. 3), leaves from higher nodes (16–18) developed few lesions in response to 3 acid rain applications as opposed to leaves on nodes 5–7 for the initial 3 sprays. This may indicate a seedling tolerance of acid rain at pH 2.50 for younger leaves on more mature seedlings. These observations were similar to those reported for other woody plants (3, 4, 19).

Significant lesion induction occurred at pH 2.25 and pH 2.50 (Table 2). Growth was reduced 25% at pH 2.25 in comparison to the control after 3 acid rain applications. There was also a trend toward growth reduction at pH 2.50. These results concur with those reported for sugar maple and yellow birch where growth reduction occurred at pH 2.3 (3, 14, 15). The threshold for vegetative growth reduction in apples was at a lower pH level than for soybeans (3, 5). The negative relationship between percentage of leaf lesions and total dry weight, after 3 treatments with a correlation coefficient of −0.444, was not significant (Fig. 4). This relationship became significant as plant age and

Table 1. Leaf lesion development, total dry weight, and leaf area of greenhouse-grown *M. hupehensis* seedlings after 9 weekly acid rain treatments (destructive harvest 3, Sept. 1–Dec. 7, 1981).

pH	Acid rain necrosis (% leaf area)	Leaf area/ plant (cm^2)	Total dry wt/ plant (g)
2.25	20.3 a[z]	358 e	3.7 f
2.50	7.8 b	532 d	5.9 e
2.75	0.9 c	569 cd	7.0 abc
3.00	0.4 c	594 bc	6.6 cdc
3.25	0.1 c	599 abc	6.7 bcd
3.50	0 c	614 abc	7.0 abc
3.75	0 c	662 a	7.5 a
4.00	0 c	647 ab	7.4 ab
4.50	0 c	610 abc	6.8 abcd
5.60	0 c	572 cd	6.1 de
7.00	0 c	620 abc	7.4 ab
Unsprayed control	0 c	614 abc	6.8 abcd

[z]Means separated within columns by Waller-Duncan's BSD test, k = 100.

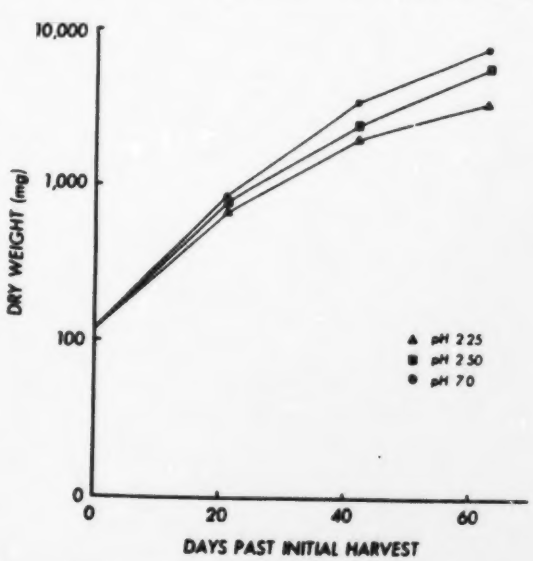

Fig. 1. Growth (log scale) of *M. hupehensis* seedlings treated with simulated acid rain solutions Oct. 5, 1981 (pre-acid rain application; 8-node stage and initial destructive harvest) - Dec. 7, 1981 (after 9 weekly acid rain applications). Expressed as means of total seedling dry weight over time.

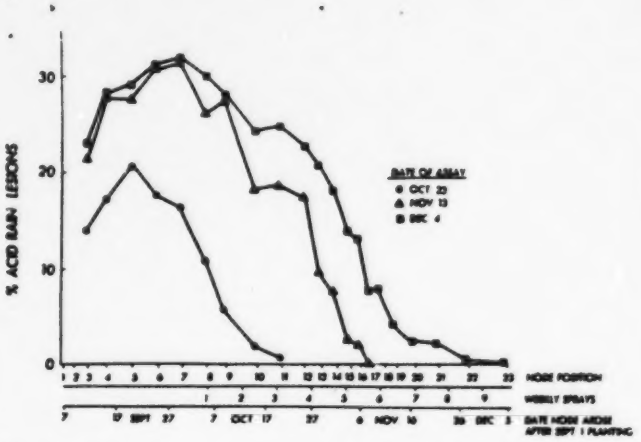

Fig. 2. Time-course development of acid rain leaf lesions on *M. hupehensis* seedlings at specific nodes after treatment with acid rain at pH 2.25. Data points are means of the same 14 plants at 3 different assay dates. Visual determinations of leaf necrosis (assays) were made 4 days after the 3rd, 6th, and 9th weekly sprays.

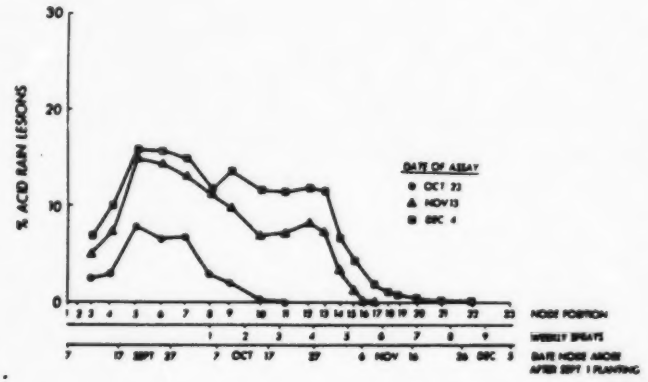

Fig. 3. Time-course development of acid rain leaf lesions on *M. hupehensis* seedlings at specific nodes after treatment with acid rain at pH 2.50. Data points are means of the same 14 plants at 3 different assay dates. Visual determinations of leaf necrosis (assays) were made 4 days after the 3rd, 6th, and 9th weekly sprays.

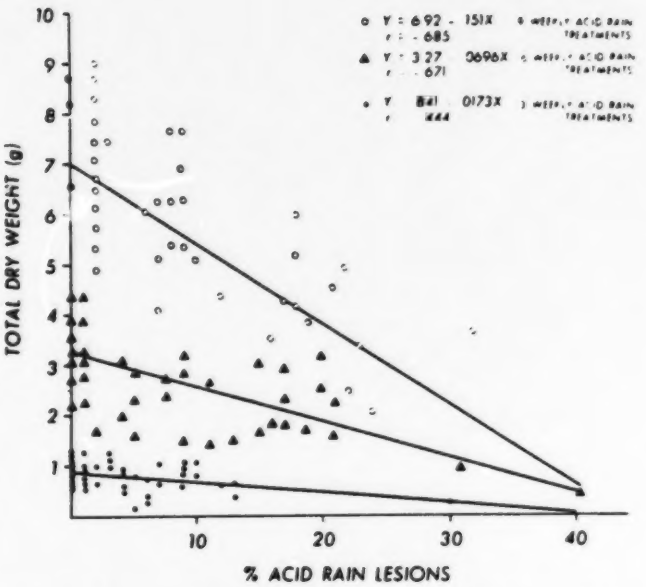

Fig. 4. Relationship between leaf necrosis and total *M. hupehensis* seedling dry weight at 3 destructive harvests after treatment with simulated acid rain. Data from pH treatments 2.25, 2.50, 2.75, 3.0, and 3.25 (the range in which visible lesions were induced).

number of acid rain applications increased (Fig. 4).

Similar lesion induction occurred at pH 2.25 and pH 2.50, with significant growth reduction also at pH 2.50 after 6 acid rain applications (Table 3). Growth reduction in comparison to control was 44% at pH 2.25 and 31% at pH 2.50. Growth was not significantly reduced at other treatment levels. The negative relationship between growth reduction and lesion formation after 6 acid rain applications (correlation coefficient of -0.671) is shown in Fig. 4.

Leaf lesion induction for whole seedlings stabilized between harvest 2 and harvest 3 (Table 1 and Table 3). Significant lesion induction was observed at pH 2.25 and 2.50, with significant growth reduction of 46% at pH 2.25 in comparison to the control. Growth reduction at pH 2.50 after 9 acid rain applications was

Table 2. Leaf lesion development, total dry weight, and leaf area of greenhouse-grown *M. hupehensis* seedlings after 3 weekly acid rain treatments (destructive harvest 1, Sept. 1–Oct. 26, 1981).

pH	Acid rain necrosis (% leaf area)	Leaf area/ plant (cm^2)	Total dry wt/ plant (g)
2.25	11.1 a[z]	110 d	0.67 d
2.50	4.5 b	141 c	0.78 c
2.75	0.6 c	166 ab	0.85 bc
3.00	0.2 c	145 bc	0.78 c
3.25	0 c	171 a	0.88 ab
3.50	0 c	160 abc	0.89 ab
3.75	0 c	173 a	0.94 a
4.00	0 c	170 a	0.87 ab
4.50	0 c	152 abc	0.82 bc
5.60	0 c	157 abc	0.81 bc
7.00	0 c	154 abc	0.81 bc
Unsprayed control	0 c	165 ab	0.89 ab

[z]Means separated within columns by Waller-Duncan's BSD test, k = 100.

Table 3. Leaf lesion development, total dry weight, and leaf area of greenhouse-grown *M. hupehensis* seedlings after 6 weekly acid rain treatments (destructive harvest 2, Sept. 1–Nov. 16, 1981).

pH	Acid rain necrosis (% leaf area)	Leaf area/ plant (cm^2)	Total dry wt/ plant (g)
2.25	20.9 a[z]	224 c	1.9 c
2.50	8.4 b	291 b	2.4 b
2.75	1.1 c	354 ab	3.2 a
3.00	0.3 c	361 a	3.4 a
3.25	0 c	416 a	3.7 a
3.50	0 c	370 a	3.2 a
3.75	0 c	355 ab	3.1 a
4.00	0 c	366 a	3.1 a
4.50	0 c	373 a	3.2 a
5.60	0 c	387 a	3.2 a
7.00	0 c	385 a	3.5 a
Unsprayed control	0 c	400 a	3.5 a

[z]Means separated within columns by Waller-Duncan's BSD test, k = 100.

not as apparent as after 6 acid rain applications (Table 3), indicating growth recovery by the plant from leaf injury as high as 8% of the total plant at increasing age. The negative relationship between lesion percentage and dry weight with a correlation coefficient of −0.685 (9 acid rain applications) is shown in Fig. 4.

With regression analysis (Fig. 5) relating pH to total dry weight, we observed a familiar trend as indicated by other researchers (13, 20). Growth inhibition observed from pH 2.25 to 3.0 was not observed between pH 3.25 and 4.0 after each of the 3 destructive harvests. The highest peak was most apparent after 9 acid rain applications at pH 3.75. This 'U-shaped' curve may be due to the increased availability of sulfur and nitrogen to the leaves in the pH range of 3.75 (13). Growth inhibition similar to that between pH 2.75 and 3.0 is observed again at pH 4.25–5.6. Shriner (20) determined that the noninhibitory effects in comparison to pH 7.0 control were not related to acid solutions in the soil but were due to direct foliar effects. Therefore, we decided that covers over the soil during spraying were not necessary to separate the foliar effects of acid rain on apple seedlings from soil effects. Very similar regression curves were obtained for leaf area vs. pH.

The correlation coefficient of −0.72 between photosynthesis rate and percentage of lesions (Fig. 6) was very similar to the correlation between dry weight and percentage of lesions (Fig. 4). However, 16% lesions caused an 18% reduction in Pn (Fig. 6), while 20% lesions caused a 44% reduction in dry weight (Fig. 4) after 6 weekly acid rain treatments. Photosynthesis was measured after 5 weekly acid rain treatments, but this comparison to destructive harvest after 6 weekly acid rain treatments seems valid. Growth reduction, in part, probably occurred as a result of insufficient healthy leaf tissue. Acid-induced growth reduction in soybeans occurred in the complete absence of necrotic leaf tissue (5).

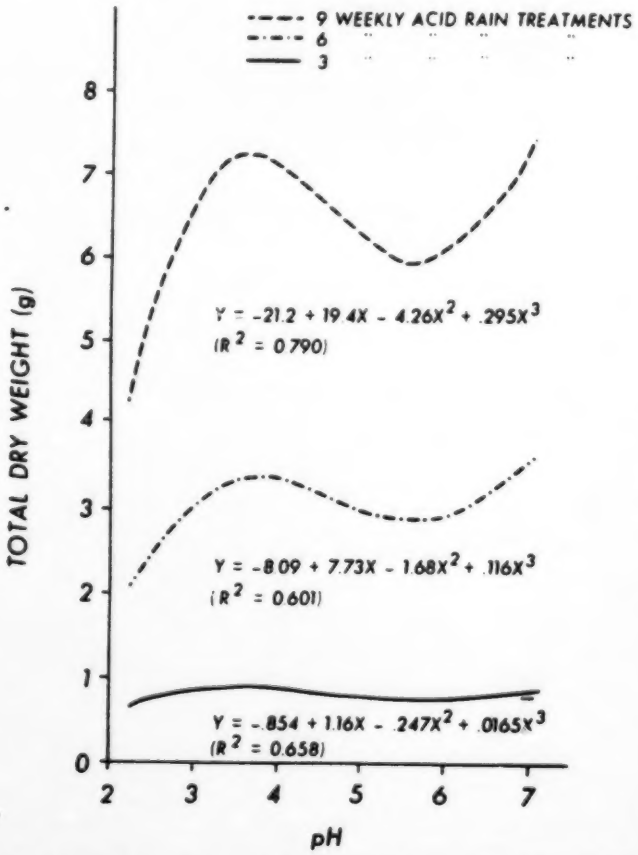

Fig. 5. Relationship between pH treatment level (simulated acid rain at pH 2.25, 2.5, 2.75, 3.0, 3.25, 3.5, 3.75, 4.0, 4.5, 5.6 and 7.0) and total *M. hupehensis* seedling dry weight at 3 destructive harvests.

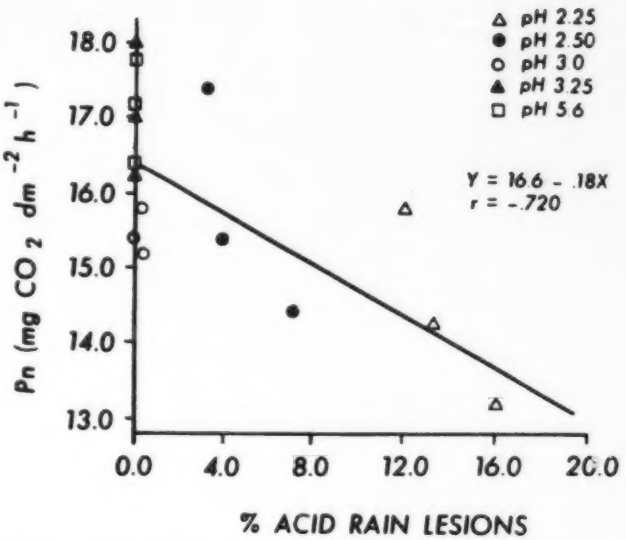

Fig. 6. Relationship between photosynthesis rate of *M. hupehensis* leaves from 3 seedlings of 5 pH treatment levels after 5 weekly acid rain applications and percentage of leaf area necrotic.

There was little change in unit leaf rate (2) as measured by the total weight gained per plant per day per cm^2 of leaf area (Table 4) during the initial 3-week acid rain application period (row 1). For the overall treatment period (row 3), pH 2.25 and pH 2.50 reduced growth compared to the other treatments. Most growth reduction at pH 2.25 occurred during the third 3-week application period (row 6). Growth reduction at pH 2.50, in comparison to higher pH treatments, was most apparent during the second 3-week application period (row 4). During the third 3-week application period (row 6), no growth reduction at pH 2.50 was evident in comparison to the control, indicating some recovery ability of an older seedling. Differences during 6-week application periods (rows 2 and 5) were apparent but not as revealing as the overall treatment period (row 3) or single 3-week periods (rows 1, 4, and 6). Effects of pH 2.75 and control treatments were similar during most periods, as were all other treatments not listed in Table 4. However, some growth inhibition at pH 2.75 is indicated in the second 3-week application period (row 4).

Relative growth rates (2), based on the assumption of logarithmic growth rate, indicate deviation from logarithmic rate even in the control (Table 5). This was explained previously with decreasing natural light levels at the end of the treatment period. Comparisons between treatments at specific periods show that the pH 2.25 treatment reduced the growth rate in all periods (rows 1–6). Again, growth rate for pH 2.50 treatment, in comparison to seedlings treated at pH 2.75 and pH 7.00, was reduced most during the second 3-week application period (row 4). Relative growth rate of plants treated with pH 2.50 solution was not reduced during the third 3-week application period (row 6). Relative growth rates of all other treatments, including those not

Table 4. Unit leaf rate (E)[y] of greenhouse-grown *M. hupehensis* seedlings after weekly acid rain applications (1981).

Acid rain application interval	Unit leaf rate (E)			
	pH 2.25	pH 2.50	pH 2.75	pH 7.00
1. Initial harvest–Harvest 1 Oct. 5–Oct. 26	0.36[z]	0.36	0.35	0.35
2. Initial harvest–Harvest 2 Oct. 5–Nov. 16	0.33	0.34	0.37	0.38
3. Initial harvest–Harvest 3 Oct. 5–Dec. 7	0.28	0.32	0.35	0.36
4. Harvest 1–Harvest 2 Oct. 26–Nov. 16	0.36	0.36	0.42	0.46
5. Harvest 1–Harvest 3 Oct. 26–Dec. 7	0.30	0.36	0.39	0.41
6. Harvest 2–Harvest 3 Nov. 16–Dec. 7	0.26	0.39	0.39	0.38

$$E = \frac{\text{Total dry wt}_2 - \text{Total dry wt}_1}{(\text{Leaf area}_1 + \text{Leaf area}_2/2)(\text{Time}_2 - \text{Time}_1)}$$

[z]Data derived from mean of 14 plants at appropriate harvest time (statistical significance cannot be determined plant by plant); dry wt = mg/cm^2 day. Initial harvest (seedlings at pre-acid rain treatment); Harvest 1 (seedlings after 3 weekly acid rain treatments); Harvest 2 (seedlings after 6 weekly acid rain treatmenis); Harvest 3 (seedlings after 9 weekly acid rain treatments).

Table 5. Relative growth rate (R)[y] of greenhouse-grown *M. hupehensis* seedlings after weekly acid rain applications (1981).

Acid rain application interval	Relative growth rate (R)			
	pH 2.25	pH 2.50	pH 2.75	pH 7.00
1. Initial harvest–Harvest 1 Oct. 5–Oct. 26	0.57[z]	0.63	0.66	0.64
2. Initial harvest–Harvest 2 Oct. 5–Nov. 16	0.46	0.50	0.55	0.56
3. Initial harvest–Harvest 3 Oct. 5–Dec. 7	0.37	0.43	0.45	0.46
4. Harvest 1–Harvest 2 Oct. 26–Nov. 16	0.35	0.38	0.44	0.49
5. Harvest 1–Harvest 3 Oct. 26–Dec. 7	0.27	0.34	0.35	0.37
6. Harvest 2–Harvest 3 Nov. 16–Dec. 7	0.19	0.29	0.27	0.25

$$R = \frac{\text{Log}_e \text{ total dry wt}_2 - \text{Log}_e \text{ total dry wt}_1}{\text{Time}_2 - \text{Time}_1}$$

[z]Data derived from mean of 14 plants at appropriate harvest time (statistical significance cannot be determined plant by plant); Log$_e$ dry wt = mg/week. Initial harvest (seedlings at pre-acid rain treatment); Harvest 1 (seedlings after 3 weekly acid rain treatments); Harvest 2 (seedlings after 6 weekly acid rain treatments); Harvest 3 (seedlings after 9 weekly acid rain treatments).

listed, revealed slight differences for particular application periods. At pH 2.75, growth rate in comparison to the control was reduced only during the second 3-week application period (row 4). The differences that occurred during this period (Oct. 26–Nov. 16) reveal that acid rain affected growth rate maximally during the period of maximum seedling growth. Maximum growth rates are also indicated in Fig. 3 and Fig. 4 with new nodal-development rates greatest between Oct. 26 and Nov. 16.

In conclusion, the threshold of growth reduction in *M. hupehensis* seedlings from acid rain was shown to be between pH 2.5 and pH 2.75, which corresponds closely to the threshold for significant leaf injury. Correlations between growth and leaf necrosis confirm the use of lesion rating in some plants as an estimator of physiological damage. Further study is needed to correlate the effects of acid rain on *M. hupehensis* with fruiting apple cultivars in orchards. Obviously, such a threshold of growth reduction is well below current pH levels of ambient rainfall in the northeastern United States (11).

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